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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/520,538	03/08/2000	Arlene A. Wise	S-91,714	2050

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UNIVERSITY OF CALIFORNIA
LOS ALAMOS NATIONAL LABORATORY
P.O. BOX 1663, MS A187
LOS ALAMOS, NM 87545

EXAMINER	
RAMIREZ, DELIA M	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 11/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/520,538	WISE ET AL.	
	Examiner	Art Unit	
	Delia M. Ramirez	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 September 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 26-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 26* is/are rejected.

7)☒ Claim(s) *27-29* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a)☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1)☐ Notice of References Cited (PTO-892)
2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3)☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____.
4)☐ Interview Summary (PTO-413) Paper No(s). _____.
5)☐ Notice of Informal Patent Application (PTO-152)
6)☐ Other: .

DETAILED ACTION

Status of the Application

Claims 1 and 26-29 are pending.

It is noted that the examination of the instant application has been assigned to a different Examiner in Group Art Unit 1652.

Applicant's amendment of claim 1 and addition of claims 26-29 in a communication filed on 9/2/2003 is acknowledged.

Because applicant did not distinctly and specifically point out the status of claims 9-25 either in the Remarks section or in the list of pending claims, and no presentation of said claims has been made in the list of pending claims, claims 9-25 will be treated as cancelled.

Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Priority

1. Acknowledgment is made of a claim for domestic priority under 35 U.S.C. 119(e) to provisional application No. 60/123,659 filed on 03/09/1999.

Claim Objections

2. Claims 1 and 26 are objected to because of the following informalities: for clarity, it is suggested that a comma be inserted between the terms "coli" and "in response", as well as in between the terms "phenols" and "over the transcriptional activation". Appropriate correction is required.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claim 1 remains rejected and newly added claim 26 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pavel et al. (J Bacteriol. 176:7550-7557, 1994) in view of Cadwell et al. ("Mutagenic PCR" pages 583-589 in "PCR Primer, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1995). Pavel et al. teaches a method for generating a Pseudomonas DmpR effector specificity mutant (DmpR-E135K) by chemical mutagenesis of Pseudomonas DmpR-encoding DNA (page 7551, right column to page 7552, right column). The resulting mutant DmpR-E135K has a single mutation in the DmpR sensor domain (referred to as the "amino terminal A domain" by Pavel et al., page 7556, left column, second paragraph) with no other mutations present in either of the DmpR DNA binding or the transactivation domains (page 7552, left and right columns and page 7554, left column, lines 38-41). DmpR-E135K exhibited increased transcriptional activation of a luciferase reporter gene under control of the dmpR promoter in response to the presence of 4-methylphenol, 3,4-dimethylphenol, and 4-ethylphenol

relative to wild-type DmpR while maintaining a wild-type-like response to phenol, 2-methylphenol, and 3-methylphenol (page 7554, right column, lines 4-32; Figure 3). Since DmpR-E135K and not the wild-type organism responds to 4-ethylphenol, Pavel et al. concludes that the mutant gained the ability to recognize a novel effector compound (page 7556, left column, bottom). Pavel et al. teach that the application of microbial metabolic activities to detoxification and environmental cleanup has stimulated interest in the construction of strains with improved degradative efficiencies or expanded catabolic capacities and suggest two methods for genetically manipulating strains for improved efficiency: overexpression of catabolic enzymes and creation of effector specificity mutants (page 7555, right column and page 7556, left column). Pavel et al. teach that genetic manipulation to create effector specificity mutants is likely to be more successful in the construction of strains with improved degradative properties (page 7556, left column, top). The method of Pavel et al. does not teach mutagenic PCR for mutating the DmpR sensor domain. Cadwell et al. teach a mutagenic PCR method of randomly mutating a nucleic acid in order to generate a library of mutant nucleic acids with scattered random mutations over the entire sequence (pages 583 and 584). Cadwell et al. further teach that after generating these mutants, one can apply a screening method to isolate individual clones that exhibit a particular property. (page 583). Cadwell et al. does not teach mutagenic PCR of the DmpR sensor domain.

Claims 1 and 26 are drawn to a method of enhancing transcriptional activation of a reporter gene under the control of a promoter regulated by a DmpR protein in Pseudomonas or E. coli bacteria, wherein the activation is in response to phenols and substituted phenols, relative to the transcriptional activation exhibited by the wild type Pseudomonas or E. coli bacteria, wherein said method comprises mutating DNA encoding the sensor domain of the DmpR protein by using mutagenic PCR, introducing the mutated DNA into the DNA encoding the DmpR protein, and

testing for enhanced transcriptional activation in response to said phenols and substituted phenols, without altering other domains of the DmpR protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use mutagenic PCR, as taught by Cadwell et al., in the method of Pavel et al. to obtain a DNA encoding the mutant DmpR sensor domain, introduce said mutated DNA into the DNA encoding the DmpR protein and test for enhanced transcriptional activation in response to phenols and substituted phenols. A person of ordinary skill in the art is motivated to use mutagenic PCR in the method of Pavel et al. since all the reactions are carried out in one tube, commercial kits are widely available, speed and ease, as well as the ability to use double stranded DNA templates. One of ordinary skill in the art has a reasonable expectation of success at using mutagenic PCR in the method of Pavel et al., introduce the mutated DNA into the DNA encoding the DmpR protein, and test for enhanced transcriptional activation, since PCR mediated mutagenesis and DNA manipulation techniques to introduce a specific DNA are well known and widely used in the art. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

6. This rejection has been previously applied to claim 1 by the previous Examiner of record. It is applied to newly added claim 26 and maintained for amended claim 1 for the reasons of record and those set forth above.

7. Applicants argue that a statement made by the previous Examiner of record in page 6, line 3 of Paper No. 20, mailed on 5/1/2003, appears to be factually incorrect. Applicants also argue that Pavel et al. does not teach how a mutation in the sensor domains results in the acquisition of an ability to recognize a novel effector compound and that this relevant since Pavel's results would not lead one of skill in the art to limit mutations to the sensor domain. Applicants submit that Pavel et al. only suggest that activation of DmpR and XylR is mediated by aromatic effector binding to the A domains of these regulators. Also, Applicants submit that

inventors Wise and Kuske in a 2000 publication indicated that the mechanism by which DmpR binds its chemical effectors and changes conformation for transcriptional activation is not known and that mutations that alter the effector profile of DmpR or XylR exert their effect through improvement of the effector-protein interaction or by changing the 3D structure of the protein to enhance, for example, polymerase activation. In view of these teachings, Applicants argue that there is no indication or suggestion in Pavel et al. that one should avoid mutation of other parts of the DmpR protein and that the Office's conclusion regarding the motivation to mutate only the sensor domain is in error.

In regard to the mutagenesis technique used, Applicants submit that in view of the many mutagenesis techniques known in the art at the time the invention was made, there is no suggestion in Pavel that the technique used by Applicants should be the one selected from the many techniques known. It is Applicant's opinion that one of skill in the art would be motivated to repeat Pavel's experiments to screen for additional effector specificity mutant using chemical mutagenesis. Applicants submit that the method of Cadwell et al. is one of the many methods available at the time the invention was made and that the Office has not explained why one would be drawn to mutagenic PCR. Applicants also submit that the Office has not addressed why one would not have chosen recursive sequence recombination as taught by Willardson, or gene shuffling as taught by Stemmer, over the method of Cadwell et al. According to Applicants, the Office has twice adopted the position that Stemmer teaches away from the use of mutagenic PCR, therefore using the Office's own reasoning, it is unlikely that one of skill in the art would be motivated to combine Pavel with Cadwell. Applicants submit that (1) the office has used hindsight reasoning, (2) Applicant's choice of mutagenic PCR would not have been the obvious choice of one of skill in the art, (3) there is no suggestion in Pavel that a mutagenesis technique aimed at low level single base point mutations would be desirable, (4) the explanation presented

by the Office is conclusory and as such, the Office has not met its obligation to provide a factual basis for the rejection.

8. Applicant's arguments have been fully considered but are not deemed persuasive to overcome the rejection of claim 1 or avoid the rejection of newly added claim 26. While it is agreed that Pavel et al. does not teach that they were only looking for sensor domain specific mutants, it is noted that the present Examiner has carefully analyzed the statement in page 6, line 3 of the Final Action submitted by the previous Examiner of record, and it appears that the intended meaning of the sentence is that Pavel's objective was to identify an effector specificity mutant and found a mutant wherein the mutation resided only in the sensor binding domain of the DmpR protein. As such, the statement is not deemed factually incorrect as asserted by Applicants.

The Examiner acknowledges (1) the teachings of inventors Wise and Kuske in a 2000 publication, (2) that Pavel et al. does not teach how a mutation in the sensor domain results in the acquisition of an ability to recognize a novel effectors, (3) mutations that affect the effector profile of DmpR or XylR exert their effect by improving effector-protein interaction or by changing the 3D structure of the protein to enhance, for example, polymerase activation, and (4) that Pavel et al. does not teach to avoid mutations of other parts of the DmpR protein. However, the Examiner disagrees with Applicant's contention that the Office's conclusion in regard to mutate only the sensor domain is in error. The mechanism by which mutations in the sensor domain of the DmpR protein result in the ability to recognize novel effectors, i.e. how it works, is completely irrelevant to the fact that Pavel et al. teaches that mutations in the sensor domain of the DmpR protein result in (1) enhanced transcriptional activation of the luciferase gene in response to 4-methylphenol, and 3,4-dimethylphenol, and (2) the ability to recognize a novel effector, i.e. 4-ethylphenol. The findings of Pavel et al. would be sufficient for one of skill in the art to recognize that mutations in the sensor domain of the DmpR protein are very likely to

correlate with recognition of novel effectors and/or enhance response to other phenols/substituted phenols. While it is agreed that Pavel et al. does not teach to avoid other domains in the DmpR protein and that it is possible that mutations in other domains of the DmpR protein, alone or in combination with mutations in the sensor domain of the DmpR protein, may result in an enhanced response to phenols and substituted phenols, this does not contradict the fact that a single mutation in the sensor domain of the DmpR protein as disclosed by Pavel et al. is sufficient to enhance the response to 4-methylphenol and 3,4-dimethylphenol, as well as allow the recognition of a novel effector, i.e. 4-ethylphenol.

Applicant's arguments in regard to the use of mutagenic PCR as opposed to other known mutagenesis methods known at the time the invention was made are found not persuasive. First, arguments in regard to the use of recursive sequence recombination as taught by Willardson, or gene shuffling as taught by Stemmer, over the method of Cadwell et al. are moot in view of the fact that the claims recite a limitation in regard to "mutagenic polymerase chain reaction (PCR)". While the Examiner acknowledges the advantages and disadvantages of the many mutagenesis methods, as indicated above, mutagenic PCR present many advantages, such as the availability of commercial kits, the use of double stranded DNA, and the use of a single tube to carry out the PCR reactions. In addition, there is no teaching or suggestion that a particular mutagenesis method in the method of Pavel et al. (1) should be completely avoided, or (2) would render superior results. Therefore, in the absence of any teaching or suggestion indicating that mutagenic PCR should be avoided and in the absence of any evidence indicating that this particular mutagenesis technique renders unexpected results, one of skill in the art would be motivated to use mutagenic PCR for the reasons set forth above.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so

long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

While it is agreed that (1) one could repeat Pavel's experiments to screen for additional effector specificity mutant using chemical mutagenesis, and (2) there is no suggestion in Pavel that a mutagenesis technique aimed at low level single base point mutations would be desirable, it is noted that once the teachings of Pavel were known in regard to which DmpR protein domain to mutate and even which residue to mutate, one of skill in the art would be more interested in a mutagenesis technique aimed at low level single base point mutations, since the area where the mutations are desired is now more limited. Therefore, for the reasons set forth above, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made in view of the teachings of Pavel et al. and Cadwell et al.

Allowable Subject Matter

9. Claims 27-29 appear to be allowable over the prior art of record but are objected to since they depend upon a rejected base claim.

Conclusion

10. Applicant's amendment of claim 1 and/or the addition of new claims 26-27 necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO

MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

11. Certain papers related to this application may be submitted to Art Unit 1652 by facsimile transmission. The FAX number is (703) 308-4556. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 CFR 1.6(d)). NOTE: If Applicant submits a paper by FAX, the original copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED, so as to avoid the processing of duplicate papers in the Office.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Delia M. Ramirez whose telephone number is (703) 306-0288. The examiner can normally be reached on Monday-Friday from 8:30 AM to 5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Ponnathapura Achutamurthy can be reached on (703) 308-3804. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Delia M. Ramirez, Ph.D.
Patent Examiner
Art Unit 1652

DR
November 10, 2003